SOLUBILIZATION OF A CATECHOLAMINE-SENSITIVE GUANOSINE TRIPHOSPHATASE FROM TURKEY ERYTHROCYTE MEMBRANES

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1. Introduction

Catecholamine hormone stimulation of the turkey erythrocyte adenylate cyclase involves a β-adrenergic receptor, probably associated with a guanyl nucleotide binding protein, a catecholamine-sensitive guanosine triphosphatase (GTPase) and an adenylate cyclase catalytic unit [1,2]. The understanding of the intricate regulatory mechanisms which modulate the activities of these 4 different components, requires an analytical approach involving the sequential solubilization and purification of each functional unit.

We have described [3,4] the solubilization and physical separation of the turkey erythrocyte β-adrenergic receptor from the adenylate cyclase catalytic component. A 20 000-fold purification of a pharmacological active receptor was achieved by affinity chromatography [5].

We now report the solubilization of the catecholamine sensitive GTPase from the same membrane preparations.

2. Materials and methods

2.1. *Membrane preparations*

Turkey erythrocyte plasma membranes were prepared by the method in [6] with the modifications in [5]. An av. 50 mg membrane protein was obtained from 100 ml blood. Membranes were stored for many months without loss of biological activities.

2.2. *Pretreatment of membranes*

Turkey erythrocyte membranes were incubated for 20 min at 37°C with or without 50 μM (−)-epinephrine plus 100 μM GMP [7]. The incubation medium contained 2 mM $MgCl_2$, 1 mM EGTA, 90 mM NaCl and 10 mM Tris–HCl (pH 7.4) (incubation buffer) and membrane protein at 5 mg/ml. At the end of the incubation, membranes were centrifuged at 30 000 × *g* for 10 min at 4°C and washed twice with incubation buffer. The membranes obtained after removal of the supernatant were either used for the assays or solubilized.

2.3. *Solubilization*

Membranes were suspended in 2 mM $MgCl_2$, 1 mM EGTA, 90 mM NaCl, 250 mM sucrose and 10 mM Tris–HCl (pH 7.4) and treated with digitonin as in [5]. After centrifugation at 30 000 × *g* for 30 min at 4°C, the supernatant constituted the solubilized preparation. This centrifugation procedure was routinely used since ultracentrifugation at 105 000 × *g* for 60 min did not sediment additional material.

2.4. *Assays*

Adenylate cyclase ATP pyrophosphate-lyase (cyclizing) (EC 4.6.1.1) activity was determined as in [5]. Cyclic AMP was isolated and measured as in [8].

GTPase activity was measured as in [2], except that the assays contained ~10 μg protein. The specific GTPase was calculated by subtracting from the total GTP hydrolysis, the non-specific GTP hydrolysis estimated in the presence of an excess (20 μM) of unlabeled GTP.

Protein determinations were done as in [9] using bovine serum albumin as standard.

3. Results and discussion

Turkey erythrocyte membranes contain GTPase [2,5] and adenylate cyclase [4], both stimulated by catecholamine hormones. Solubilization of the plasma membranes by the use of digitonin results in the loss of GTPase sensitivity to catecholamines and of adenylate cyclase sensitivity to catecholamines and to Gpp(NH)p. However, pretreatment of the membranes with (−)-epinephrine and GMP, followed by digitonin solubilization, results in a soluble sensitive GTPase which remains in the supernatant even after centrifugation at 105 000 × g: (−)-epinephrine amplifies the enzymic activity by 78%. Pretreatment with either (−)-epinephrine or GMP alone has no effect (table 1).

Intact membranes do not require pretreatment with GMP and (−)-epinephrine to display a catecholamine sensitive GTPase (table 1): the epinephrine stimulation is not affected by pretreatment of the membranes and equals that observed in solubilized membranes. It should be noted that solubilization procedures result in a partial purification of GTPase: digitonin extracts 53% of the enzyme activity but only 18% of total membrane protein, thus providing a 3-fold increase in specific activity (table 1). Other agonists, such as (−)-norepinephrine and (−)-isoproterenol, also stimulate the soluble GTPase, whereas the corresponding (+)-isomers and the antagonists, (−)-propranolol and (−)-alprenolol, do not (table 2).

Table 2
Effects of β-adrenergic agents on GTPase activity in pretreated erythrocyte membranes

Addition	GTPase activity	% increase caused by the ligand
None	35.4	
(−)-Epinephrine	64.6	82
(−)-Norepinephrine	65.7	86
(−)-Isoproterenol	67.6	91
(−)-Propranolol	30.8	–
(+)-Epinephrine	35.2	0
(+)-Isoproterenol	34.0	–
(±)-Alprenolol	37.5	6

Membranes were pretreated with 100 μM GMP and 50 μM (−)-epinephrine for 20 min at 37°C. GTPase activities were determined in the absence (basal) or in the presence of 100 μM ligand (except 200 μM (±)-alprenolol). Activities are expressed in pmol $^{32}P_i$ liberated . 10 min^{-1} . mg $protein^{-1}$. Values are means from 3 determinations

Digitonin-solubilized extracts from epinephrine and GMP pretreated membranes also contain adenylate cyclase which is stimulated by Gpp(NH)p but is not responsive to epinephrine (fig.1). This result agrees with that found in [7] in Lubrol PX solubilized extracts from pigeon erythrocyte membranes. Pretreatment by GMP and (−)-epinephrine uncovers the GTP-induced decrease of agonist binding affinity of the turkey erythrocyte β-adrenergic receptor. These

Table 1
Influence of membrane pretreatment on the catecholamine stimulated GTPase activity in membranes and in digitonin extracts from turkey erythrocyte membranes

Membranes pretreated with	GTPase activity			
	Membranes		Digitonin extracts	
	Basal	Catecholamine stimulated	Basal	Catecholamine stimulated
Buffer	17.1	30.7 (79%)	50.5	45.9
GMP and (−)-Epinephrine	14.1	24.7 (75%)	41.9	74.5 (79%)
GMP	–	–	44.5	50.5 (13%)
(−)-Epinephrine	–	–	46.2	48.3 (5%)

Membranes (5 mg/ml) were pretreated with 100 μM GMP and/or 50 μM (−)-epinephrine for 20 min at 37°C and washed twice. GTPase activities were determined, according to [2] with modifications [5], on membranes (11 μg membrane protein/assay) and on digitonin solubilized membranes (8 μg protein/assay), in the absence (basal) and in the presence of 100 μM (−)-epinephrine (catecholamine stimulated). Activities are expressed in pmol $^{32}P_i$ liberated . 10 min^{-1} . mg $protein^{-1}$. Results are means from 3 determinations. The values in parentheses refer to the % stimulation by epinephrine of the GTPase activity

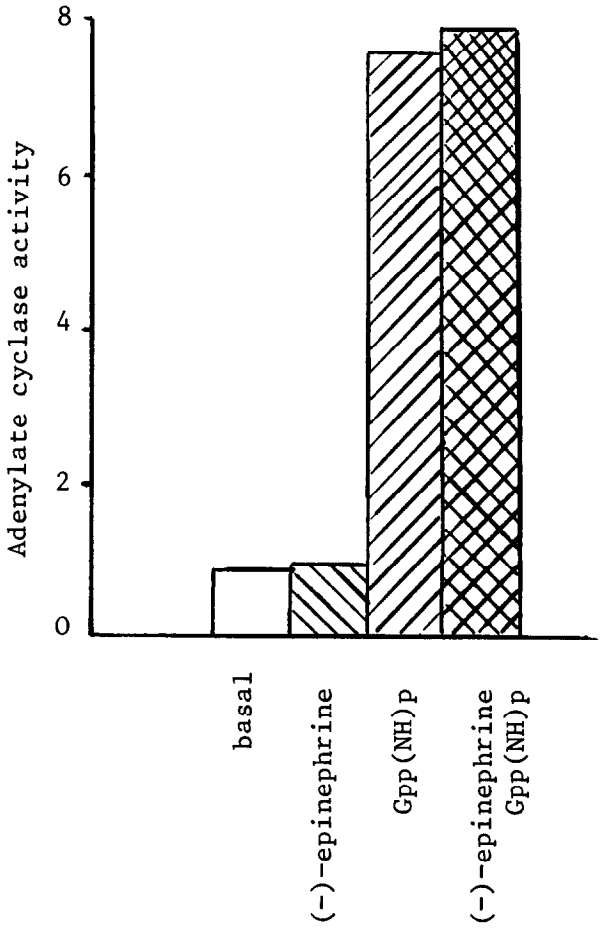

Fig.1. Adenylate cyclase activity in digitonin-solubilized extracts from GMP and epinephrine pretreated turkey erythrocyte membranes. Membranes (5 mg/ml) were pretreated with 100 μM GMP and 50 μM (−)-epinephrine for 20 min at 37°C, washed twice and solubilized by digitonin 0.25% (w/v). Adenylate cyclase activities were measured in the absence (basal) or in the presence of 100 μM (−)-epinephrine or 100 μM Gpp(NH)p or (−)-epinephrine plus Gpp(NH)p. Activities are expressed in pmol/cAMP produced . 20 min^{-1} . mg $protein^{-1}$. Values are means from 3 determinations.

findings were interpreted as removal by GMP and hormone of endogenously bound inhibitory forms of guanine nucleotides [7,10,11]. We here show that pretreatment of membranes with GMP and (−)-epinephrine apparently stabilizes, during digitonin solubilization, the catecholamine sensitivity of the turkey erythrocyte membrane GTPase. This stabilization may be explained by association in solution of the receptor and the GTPase.

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